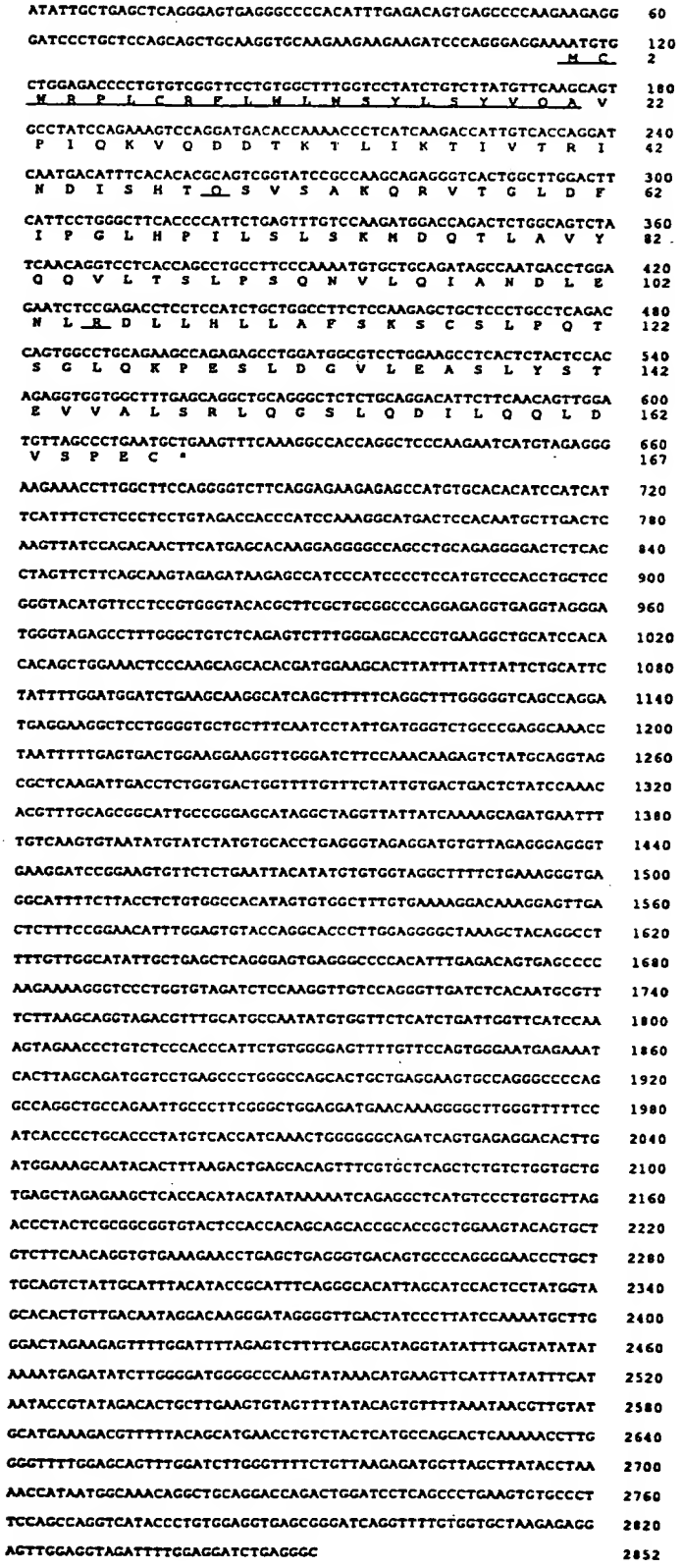
Figure 1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GATCCCTGCTCCAGCAGCTGCAAGGTGCAAGAAGAAGAAGATCCCAGGGAGGAAAATGTG 120
M C 2
CTGGAGACCCCTGTGTCGGTTCCTGTGGCTTTGGTCCTATCTGTCTTATGTTCAAGCAGT 180
W R P L C R F L W L N S Y L S Y V Q A V 22
GCCTATCCAGAAAGTCCAGGATGACACCAAAACCCTCATCAAGACCATTGTCACCAGGAT 240
P I Q K V Q D D T K T L I K T I V T R I 42
CAATGACATTTCACACACGCAGTCGGTATCCGCCAAGCAGAGGGTCACTGGCTTGGACTT 300
N D I S H T Q S V S A K Q R V T G L D F 62
CATTCCTGGGCTTCACCCCATTCTGAGTTTGTCCAAGATGGACCAGACTCTGGCAGTCTA 360
I P G L H P I L S L S K M D Q T L A V Y 82
TCAACAGGTCCTCACCAGCCTGCCTTCCCAAAATGTGCTGCAGATAGCCAATGACCTGGA 420
Q Q V L T S L P S Q N V L Q I A N D L E 102
GAATCTCCGAGACCTCCTCCATCTGCTGGCCTTCTCCAAGAGCTGCTCCCTGCCTCAGAC 480
N L R D L L H L L A F S K S C S L P Q T 122
CAGTGGCCTGCAGAAGCCAGAGAGCCTGGATGGCGTCCTGGAAGCCTCACTCTACTCCAC 540
S G L Q K P E S L D G V L E A S L Y S T 142
AGAGGTGGTGGCTTTGAGCAGGCTGCAGGGCTCTCTGCAGGACATTCTTCAACAGTTGGA 600
E V V A L S R L Q G S L Q D I L Q Q L D 162
TGTTAGCCCTGAATGCTGAAGTTTCAAAGGCCACCAGGCTCCCAAGAATCATGTAGAGGG 660
V S P E C * 167
AAGAAACCTTGGCTTCCAGGGGTCTTCAGGAGAAGAGAGCCATGTGCACACATCCATCAT 720
TCATTTCTCTCCCTCCTGTAGACCACCCATCCAAAGGCATGACTCCACAATGCTTGACTC 780
AAGTTATCCACACAACTTCATGAGCACAAGGAGGGGCCAGCCTGCAGAGGGGACTCTCAC 840
CTAGTTCTTCAGCAAGTAGAGATAAGAGCCATCCCATCCCCTCCATGTCCCACCTGCTCC 900
GGGTACATGTTCCTCCGTGGGTACACGCTTCGCTGCGGCCCAGGAGAGGTGAGGTAGGGA 960
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CACAGCTGGAAACTCCCAAGCAGCACACGATGGAAGCACTTATTTATTTATTCTGCATTC 1080
TATTTTGGATGGATCTGAAGCAAGGCATCAGCTTTTTCAGGCTTTGGGGGTCAGCCAGGA 1140
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TGTCAAGTGTAATATGTATCTATGTGCACCTGAGGGTAGAGGATGTGTTAGAGGGAGGGT 1440
GAAGGATCCGGAAGTGTTCTCTGAATTACATATGTGTGGTAGGCTTTTCTGAAAGGGTGA 1500
GGCATTTTCTTACCTCTGTGGCCACATAGTGTGGCTTTGTGAAAAGGACAAAGGAGTTGA 1560
CTCTTTCCGGAACATTTGGAGTGTACCAGGCACCCTTGGAGGGGCTAAAGCTACAGGCCT 1620
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CACTTAGCAGATGGTCCTGAGCCCTGGGCCAGCACTGCTGAGGAAGTGCCAGGGCCCCAG 1920
GCCAGGCTGCCAGAATTGCCCTTCGGGCTGGAGGATGAACAAAGGGGCTTGGGTTTTTCC 1980
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ACCCTACTCGCGGCGGTGTACTCCACCACAGCAGCACCGCACCGCTGGAAGTACAGTGCT 2220
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GGACTAGAAGAGTTTTGGATTTTAGAGTCTTTTCAGGCATAGGTATATTTGAGTATATAT 2460
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GGGTTTTGGAGCAGTTTGGATCTTGGGTTTTCTGTTAAGAGATGGTTAGCTTATACCTAA 2700
AACCATAATGGCAAACAGGCTGCAGGACCAGACTGGATCCTCAGCCCTGAAGTGTGCCCT 2760
TCCAGCCAGGTCATACCCTGTGGAGGTGAGCGGGATCAGGTTTTGTGGTGCTAAGAGAGG 2820
AGTTGGAGGTAGATTTTGGAGGATCTGAGGGC 2852

Figure 2

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---G--GTTG CAAGGCCCAA GAAGCCCA-- -TCCTGGGAA GGAAAATGCA      50
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GTCCAAGCTG TGCCCATCCA AAAAGTCCAA GATGACACCA AAACCCTCAT     150
CAAGACAATT GTCACCAGGA TCAATGACAT TTCACACACG CAGTCAGTCT     200
CCTCCAAACA GAAAGTCACC GGTTTGGACT TCATTCCTGG GCTCCACCCC     250
ATCCTGACCT TATCCAAGAT GGACCAGACA CTGGCAGTCT ACCAACAGAT     300
CCTCACCAGT ATGCCTTCCA GAAACGTGAT CCAAATATCC AACGACCTGG     350
AGAACCTCCG GGATCTTCTT CACGTGCTGG CCTTCTCTAA GAGCTGCCAC     400
TTGCCCTGGG CCAGTGGCCT GGAGACCTTG GACAGCCTGG GGGGTGTCCT     450
GGAAGCTTCA GGCTACTCCA CAGAGGTGGT GGCCCTGAGC AGGCTGCAGG     500
GGTCTCTGCA GGACATGCTG TGGCAGCTGG ACCTCAGCCC TGGGTGCTGA     550
GGCCTTGAAG GTCACTCTTC CTGCAAGGAC T-ACGTTAAG GGAAGGAACT     600
CTGGTTTCCA GGTATCTCCA GGATTGAAGA GCATTGCATG GACACCCCTT     650
ATCCAGGACT CTGTCAATTT CCCTGACTCC TCTAAGCCAC TCTTCCAAAG     700
G                                                         701
```

Figure 4

```
Mouse   MCWRPLCRFL WLWSYLSYVQ AVPIQKVQDD TKTLIKTIVT RINDISHTQS     50
Human   MHWGTLCGFL WLWPYLFYVQ AVPIQKVQDD TKTLIKTIVT RINDISHTQS

Mouse   VSAKQRVTGL DFIPGLHPIL SLSKMDQTLA VYQQVLTSLP SQNVLQIAND    100
Human   VSSKQKVTGL DFIPGLHPIL TLSKMDQTLA VYQQILTSMP SRNVIQISND

Mouse   LENLRDLLHL LAFSKSCSLP QTSGLQKPES LDGVLEASLY STEVVALSRL    150
Human   LENLRDLLHV LAFSKSCHLP WASGLETLDS LGGVLEASGY STEVVALSRL

Mouse   QGSLQDILQQ LDVSPEC                                       167
Human   QGSLQDMLWQ LDLSPGC
```

Figure 5

1	Met Cys Trp Arg Pro Leu Cys Arg Phe Leu Trp Leu Trp Ser Tyr
16	Leu Ser Tyr Val Gln Ala Val Pro Ile Gln Lys Val Gln Asp Asp
31	Thr Lys Thr Leu Ile Lys Thr Ile Val Thr Arg Ile Asn Asp Ile
46	Ser His Thr Ser Val Ser Ala Lys Gln Arg Val Thr Gly Leu Asp
61	Phe Ile Pro Gly Leu His Pro Ile Leu Ser Leu Ser Lys Met Asp
76	Gln Thr Leu Ala Val Tyr Gln Gln Val Leu Thr Ser Leu Pro Ser
91	Gln Asn Val Leu Gln Ile Ala Asn Asp Leu Glu Asn Leu Arg Asp
106	Leu Leu His Leu Leu Ala Phe Ser Lys Ser Cys Ser Leu Pro Gln
121	Thr Ser Gly Leu Gln Lys Pro Glu Ser Leu Asp Gly Val Leu Glu
136	Ala Ser Leu Tyr Ser Thr Glu Val Val Ala Leu Ser Arg Leu Gln
151	Gly Ser Leu Gln Asp Ile Leu Gln Gln Leu Asp Val Ser Pro Glu
166	Cys End

Figure 6

```
  1  Met His Trp Gly Thr Leu Cys Gly Phe Leu Trp Leu Trp Pro Tyr
 16  Leu Phe Tyr Val Gln Ala Val Pro Ile Gln Lys Val Gln Asp Asp
 31  Thr Lys Thr Leu Ile Lys Thr Ile Val Thr Arg Ile Asn Asp Ile
 46  Ser His Thr Ser Val Ser Ser Lys Gln Lys Val Thr Gly Leu Asp
 61  Phe Ile Pro Gly Leu His Pro Ile Leu Thr Leu Ser Lys Met Asp
 76  Gln Thr Leu Ala Val Tyr Gln Gln Ile Leu Thr Ser Met Pro Ser
 91  Arg Asn Val Ile Gln Ile Ser Asn Asp Leu Glu Asn Leu Arg Asp
106  Leu Leu His Val Leu Ala Phe Ser Lys Ser Cys His Leu Pro Trp
121  Ala Ser Gly Leu Glu Thr Leu Asp Ser Leu Gly Gly Val Leu Glu
136  Ala Ser Gly Tyr Ser Thr Glu Val Val Ala Leu Ser Arg Leu Gln
151  Gly Ser Leu Gln Asp Met Leu Trp Gln Leu Asp Leu Ser Pro Gly
166  Cys End
```

Figure 7

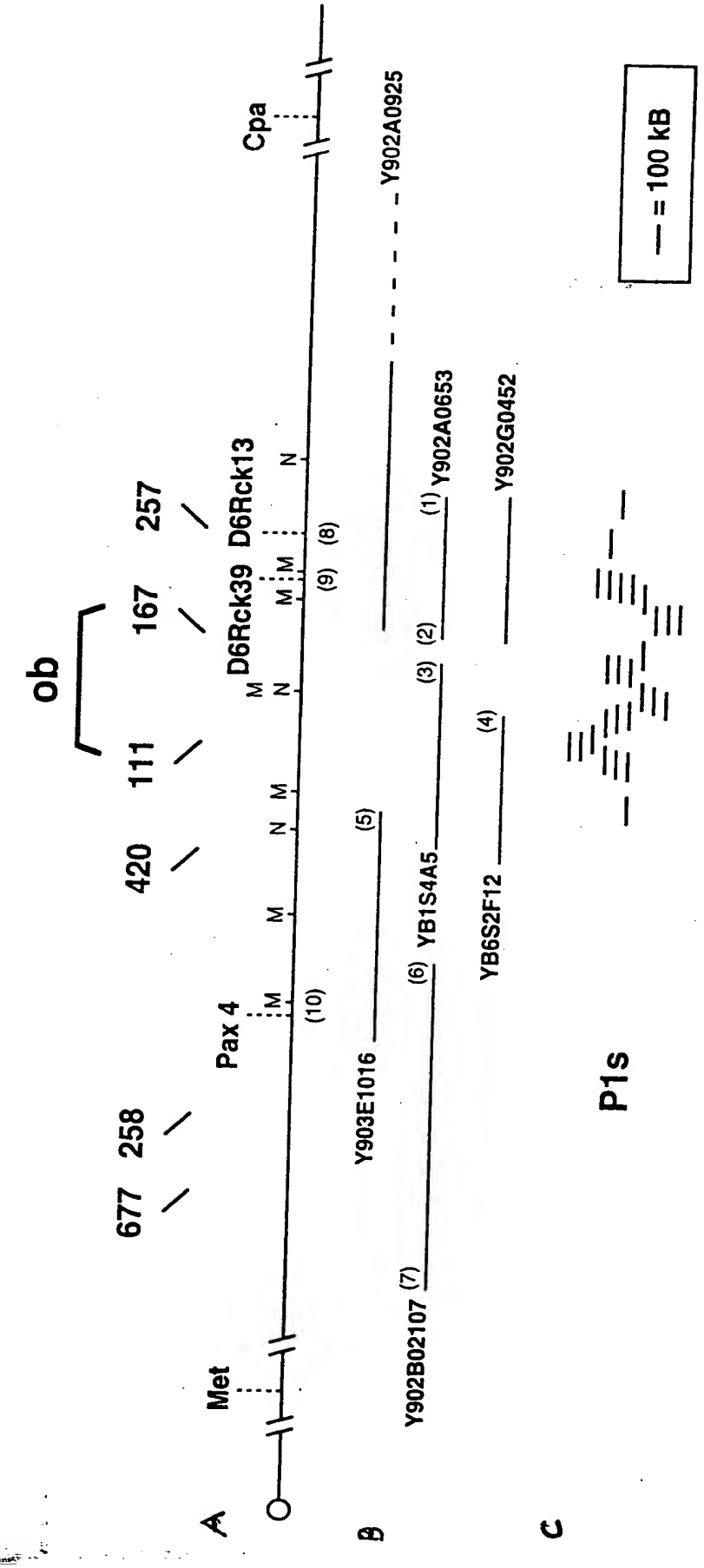

A
B
C
Met
Pax 4
677
258
420
111
ob
167
257
Cpa
M
N
M
N
M
N
M
M
M
D6Rck39
D6Rck13
(10)
(9)
(8)
Y902A0925
(1) Y902A0653
(2)
(3)
(4)
(5)
(6)
(7)
Y903E1016
YB1S4A5
YB6S2F12
Y902G0452
Y902B02107
P1s
— = 100 kB

Figure 8

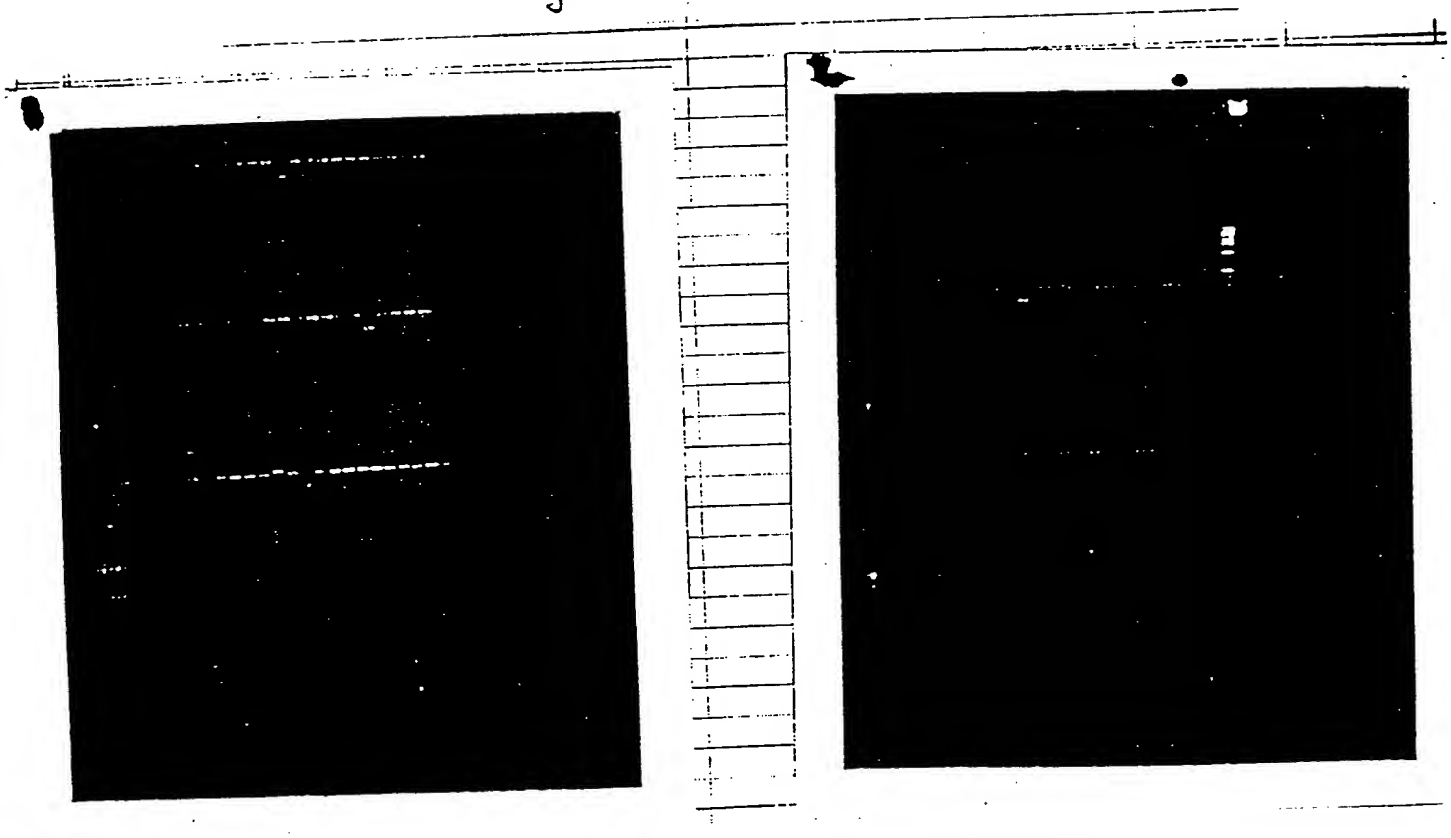


Figure 9

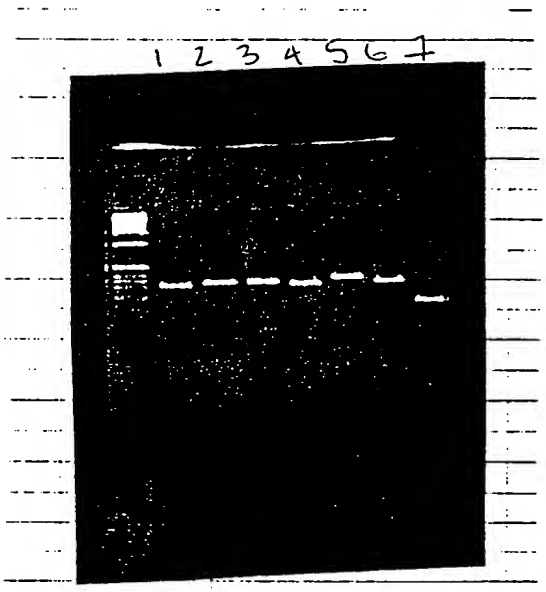
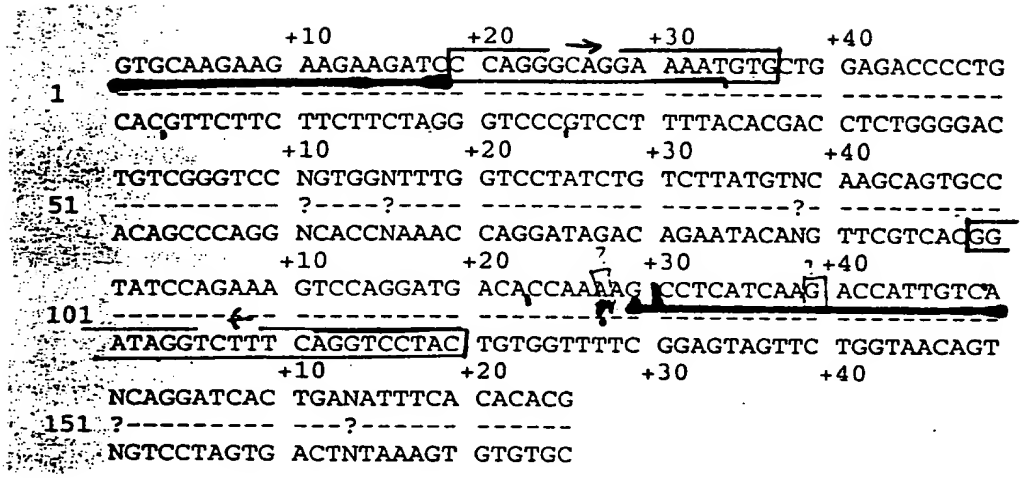


Figure 10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GTGCAAGAAG AAGAAGATCC CAGGGCAGGA AAATGTGCTG GAGACCCCTG
1
CACGTTCTTC TTCTTCTAGG GTCCCGTCCT TTTACACGAC CTCTGGGGAC
+10 +20 +30 +40
TGTCGGGTCC NGTGGNTTTG GTCCTATCTG TCTTATGTNC AAGCAGTGCC
51
ACAGCCCAGG NCACCNAAAC CAGGATAGAC AGAATACANG TTCGTCACGG
+10 +20 +30 +40
TATCCAGAAA GTCCAGGATG ACACCAAAAG CCTCATCAAG ACCATTGTCA
101
ATAGGTCTTT CAGGTCCTAC TGTGGTTTTC GGAGTAGTTC TGGTAACAGT
+10 +20 +30 +40
NCAGGATCAC TGANATTTCA CACACG
151
NGTCCTAGTG ACTNTAAAGT GTGTGC

Figure 11A

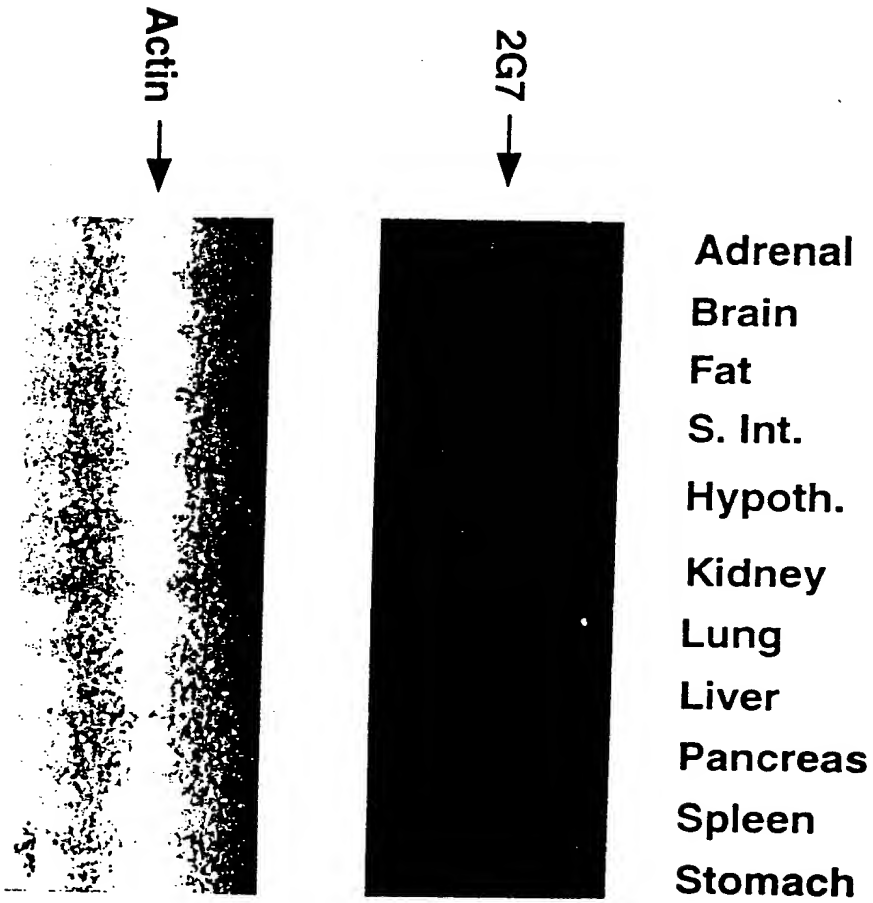

Actin
2G7
Adrenal
Brain
Fat
S. Int.
Hypoth.
Kidney
Lung
Liver
Pancreas
Spleen
Stomach

Figure 11B

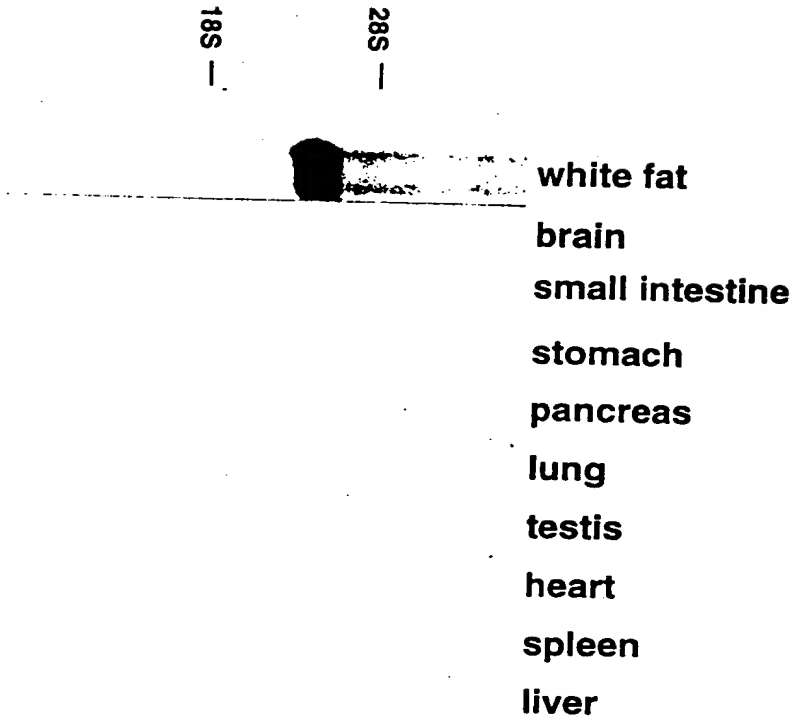

18S
28S
white fat
brain
small intestine
stomach
pancreas
lung
testis
heart
spleen
liver

Figure 12A

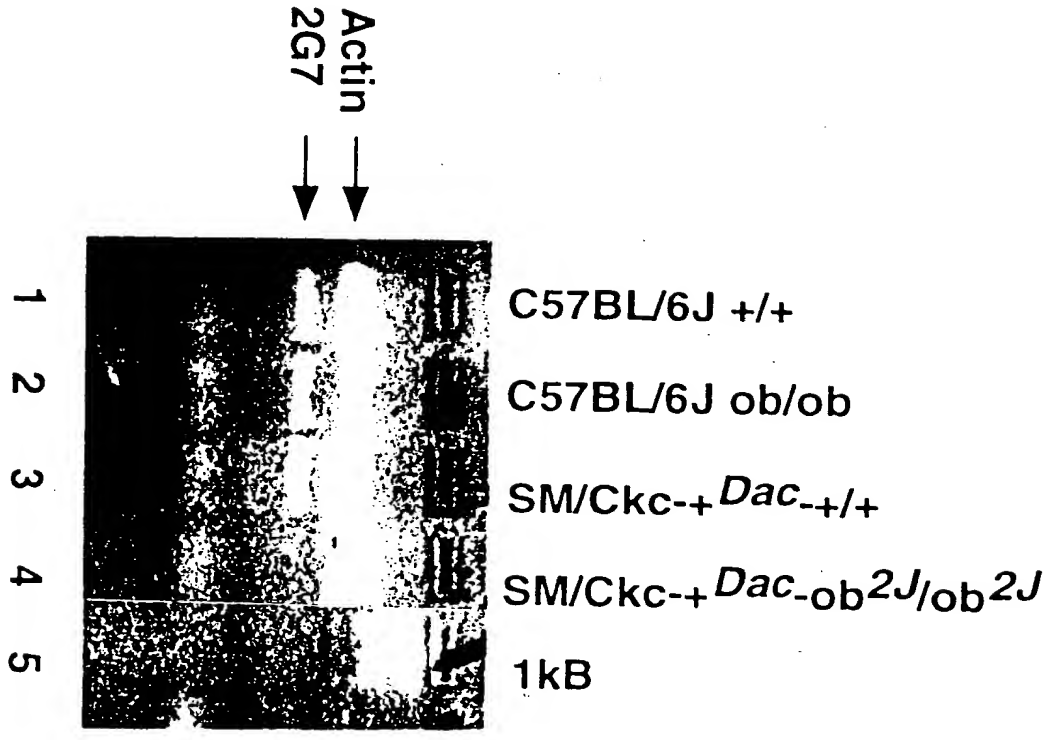

Actin
2G7
1 2 3 4 5
C57BL/6J +/+
C57BL/6J ob/ob
SM/Ckc-+Dac-+/+
SM/Ckc-+Dac-ob2J/ob2J
1kB

Figure 12 B

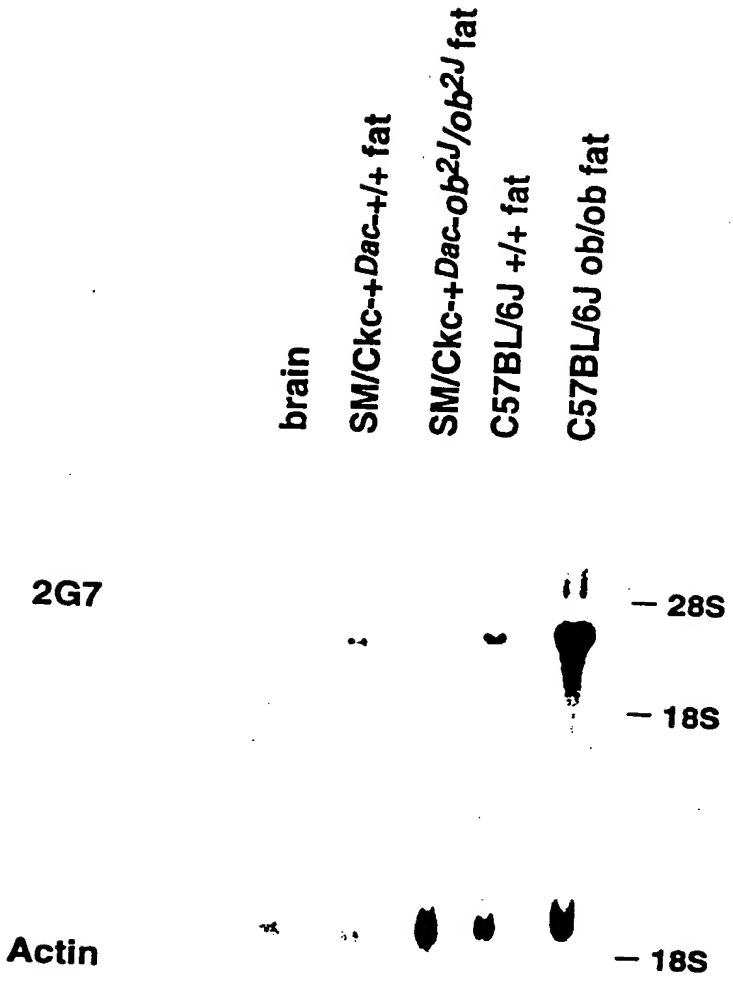

brain
SM/Ckc-+Dac-+/+ fat
SM/Ckc-+Dac-ob2J/ob2J fat
C57BL/6J +/+ fat
C57BL/6J ob/ob fat
2G7
28S
18S
Actin
18S

Figure 13

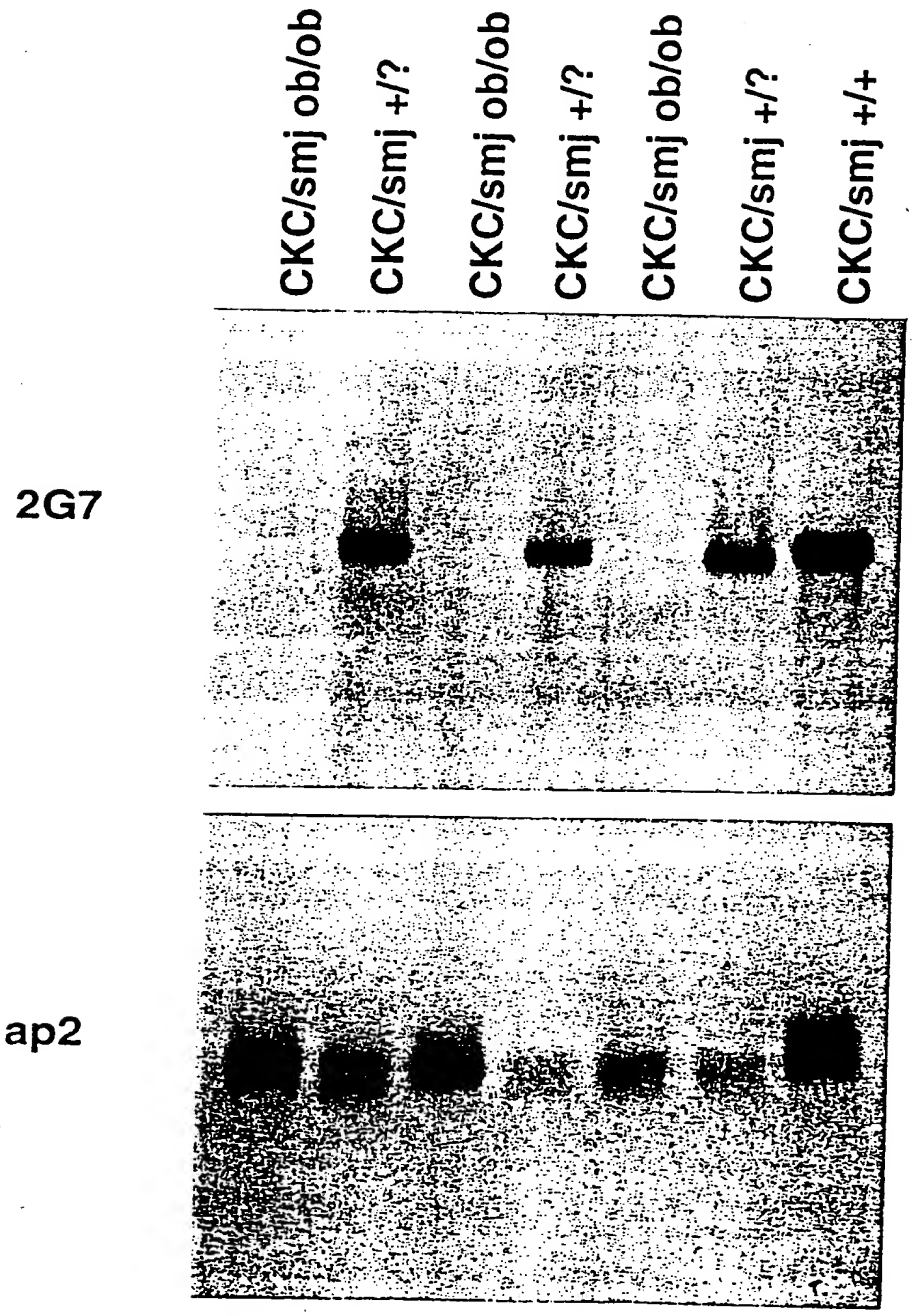

CKC/smj ob/ob
CKC/smj +/?
CKC/smj ob/ob
CKC/smj +/?
CKC/smj ob/ob
CKC/smj +/?
CKC/smj +/+
2G7
ap2

Figure 14

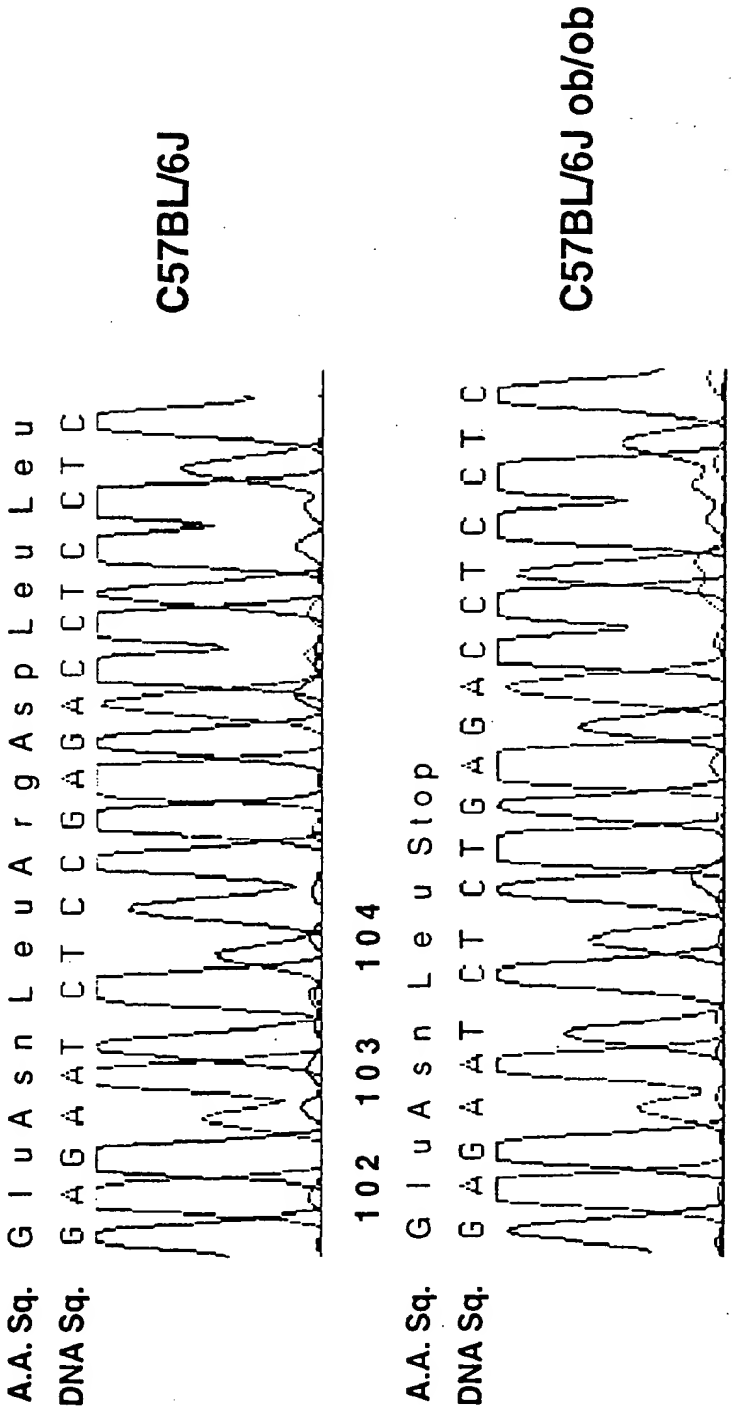

C57BL/6J
A.A. Sq.
DNA Sq.
102 103 104 105 106 107 108
Glu Asn Leu Arg Asp Leu Leu
GAG AAT CTC CGA GAC CTC CTC
C57BL/6J ob/ob
A.A. Sq.
DNA Sq.
102 103 104
Glu Asn Leu Stop
GAG AAT CTC TGA GAC CTC CTC

Figure 15A

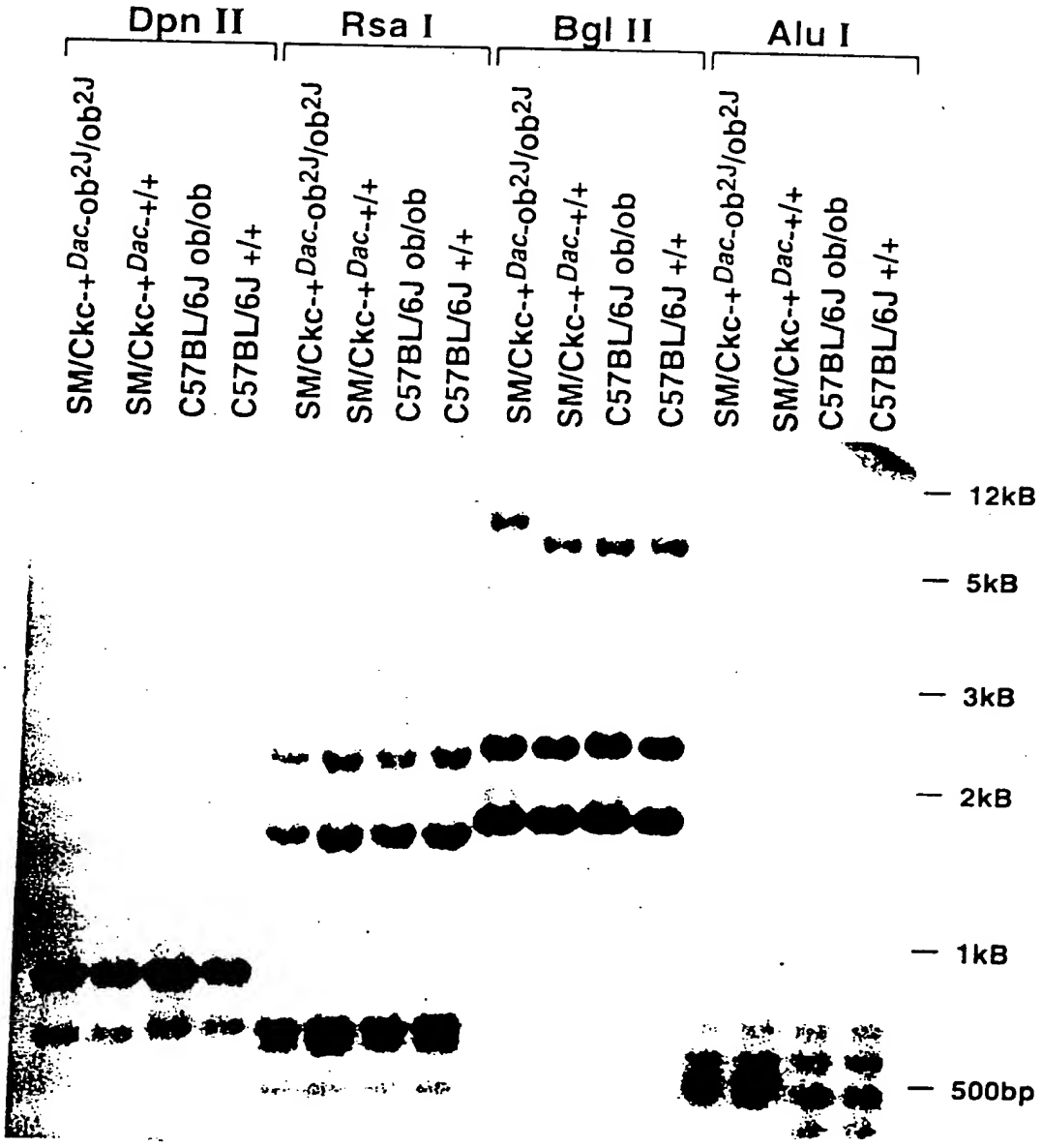

Dpn II
Rsa I
Bgl II
Alu I
SM/Ckc-+Dac-ob2J/ob2J
SM/Ckc-+Dac-+/+
C57BL/6J ob/ob
C57BL/6J +/+
SM/Ckc-+Dac-ob2J/ob2J
SM/Ckc-+Dac-+/+
C57BL/6J ob/ob
C57BL/6J +/+
SM/Ckc-+Dac-ob2J/ob2J
SM/Ckc-+Dac-+/+
C57BL/6J ob/ob
C57BL/6J +/+
SM/Ckc-+Dac-ob2J/ob2J
SM/Ckc-+Dac-+/+
C57BL/6J ob/ob
C57BL/6J +/+
12kB
5kB
3kB
2kB
1kB
500bp

Figure 16

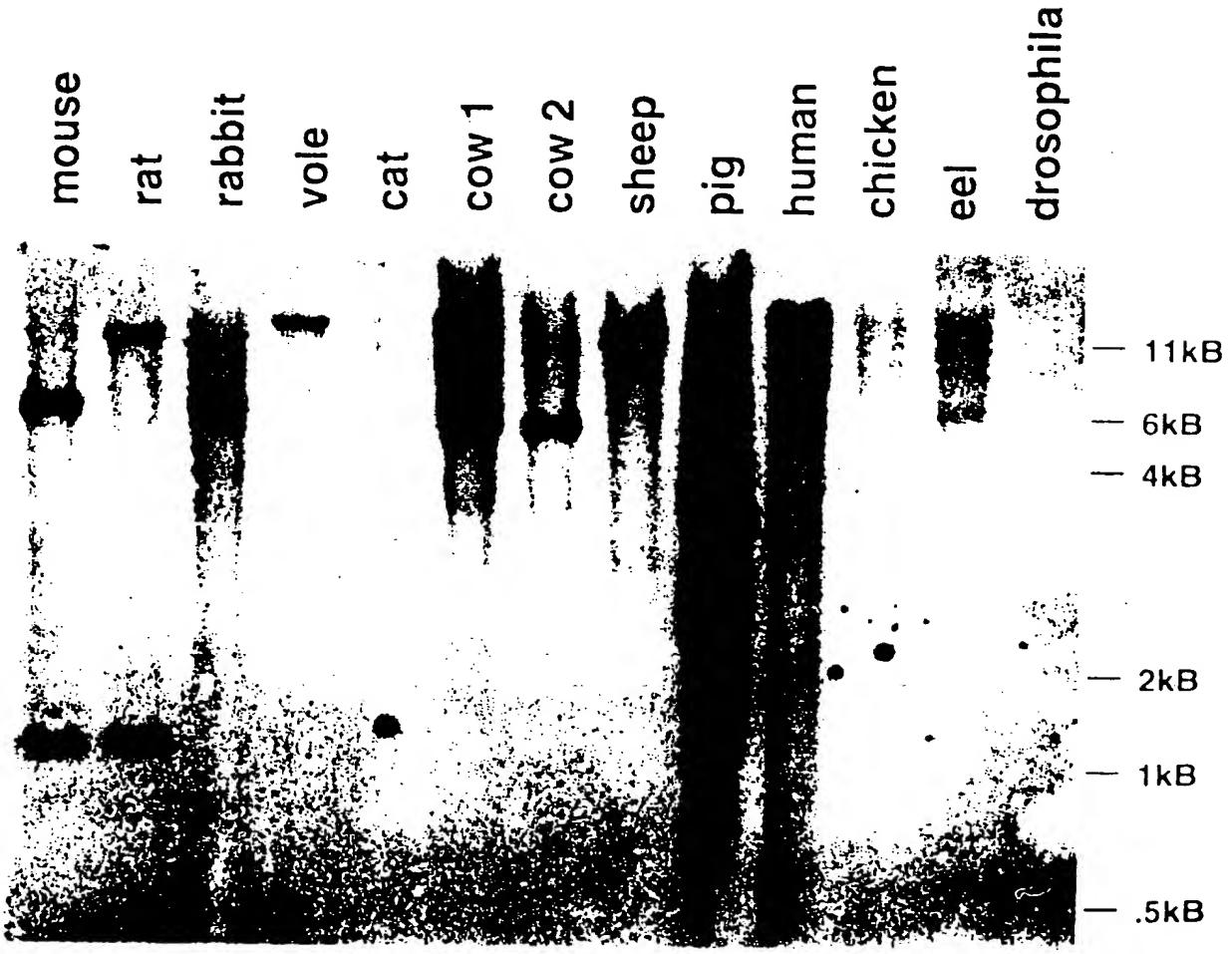

mouse
rat
rabbit
vole
cat
cow 1
cow 2
sheep
pig
human
chicken
eel
drosophila
11kB
6kB
4kB
2kB
1kB
.5kB

Figure 15B

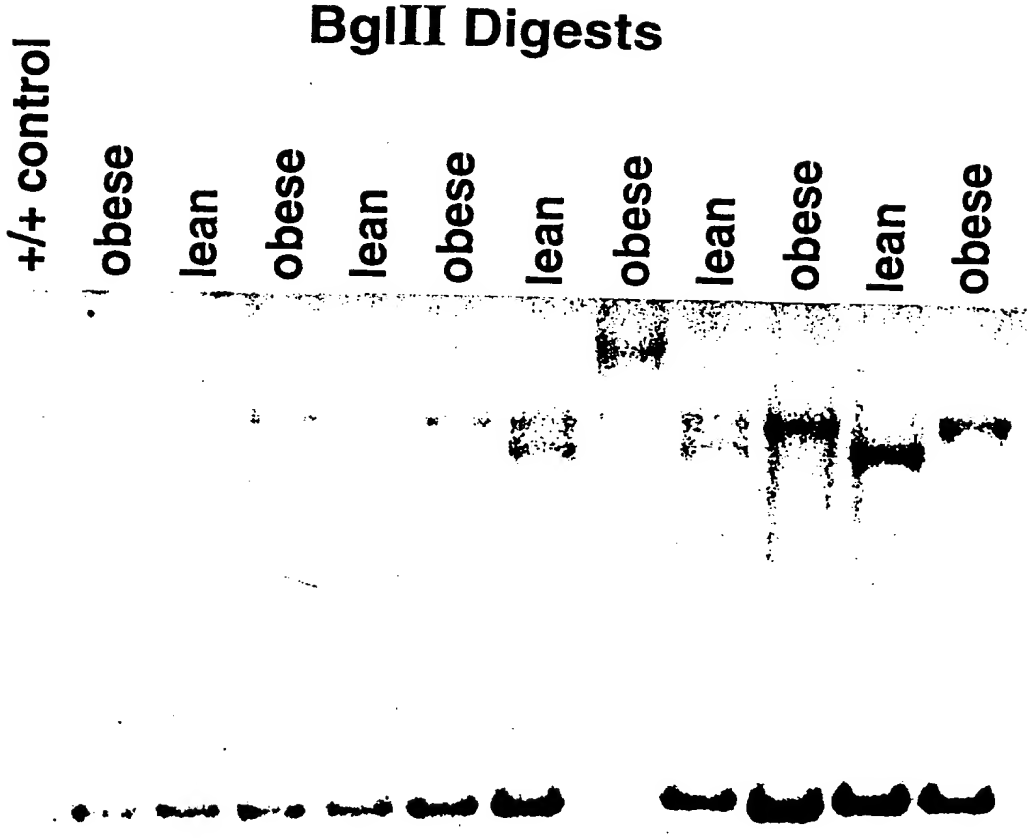

BglII Digests
+/+ control
obese
lean
obese
lean
obese
lean
obese
lean
obese
lean
obese

Figure 17

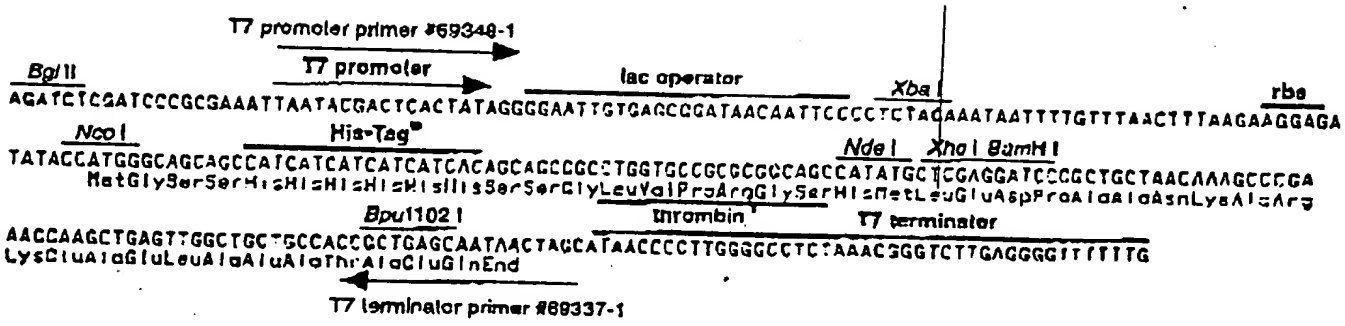

T7 promoter primer #69348-1
T7 promoter
lac operator
Bgl II
Xba I
rbs
Nco I
His-Tag
Nde I
Xho I BamH I
Bpu1102 I
thrombin
T7 terminator
T7 terminator primer #69337-1

Figure 18A

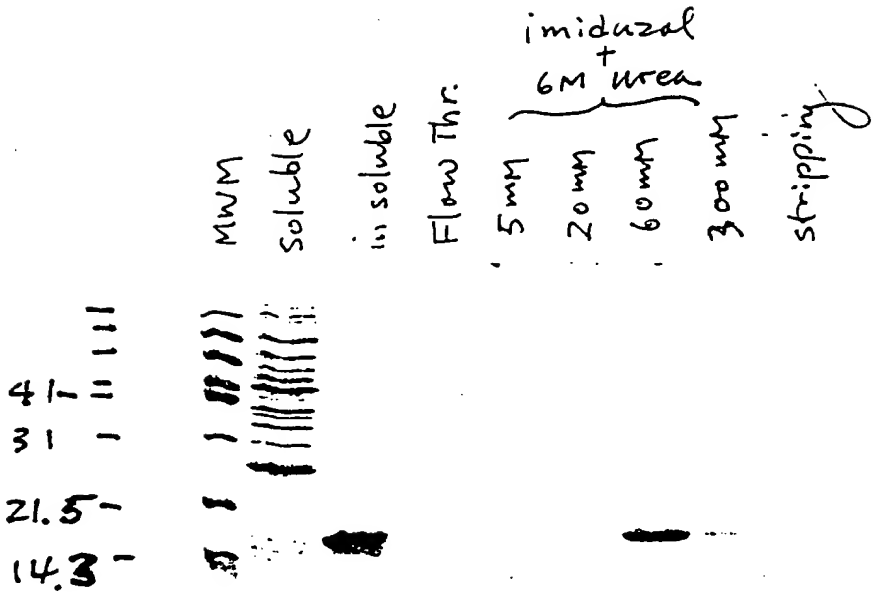
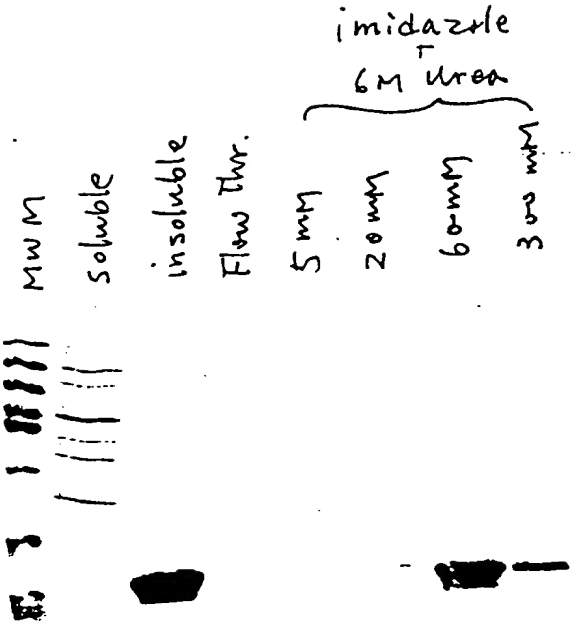

MWM
Soluble
insoluble
Flow Thr.
imidazol + 6M urea
5mM
20mM
60mM
300mM
stripping
41
31
21.5
14.3

Figure 18B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soluble
insoluble
Flow Thr.
5 mM
20 mM
60 mM
300 mM
imidazole
6M urea

Figure 19A

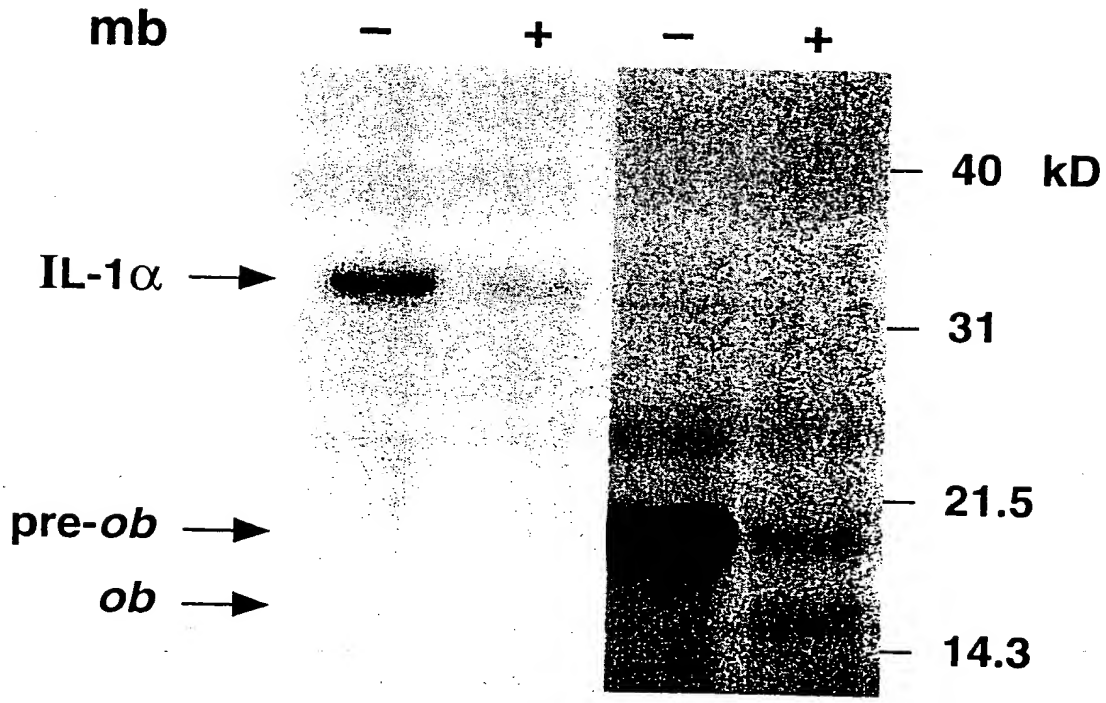


Figure 19B

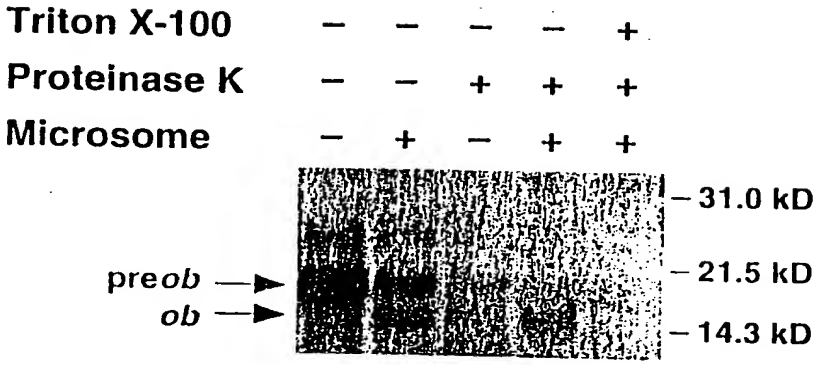
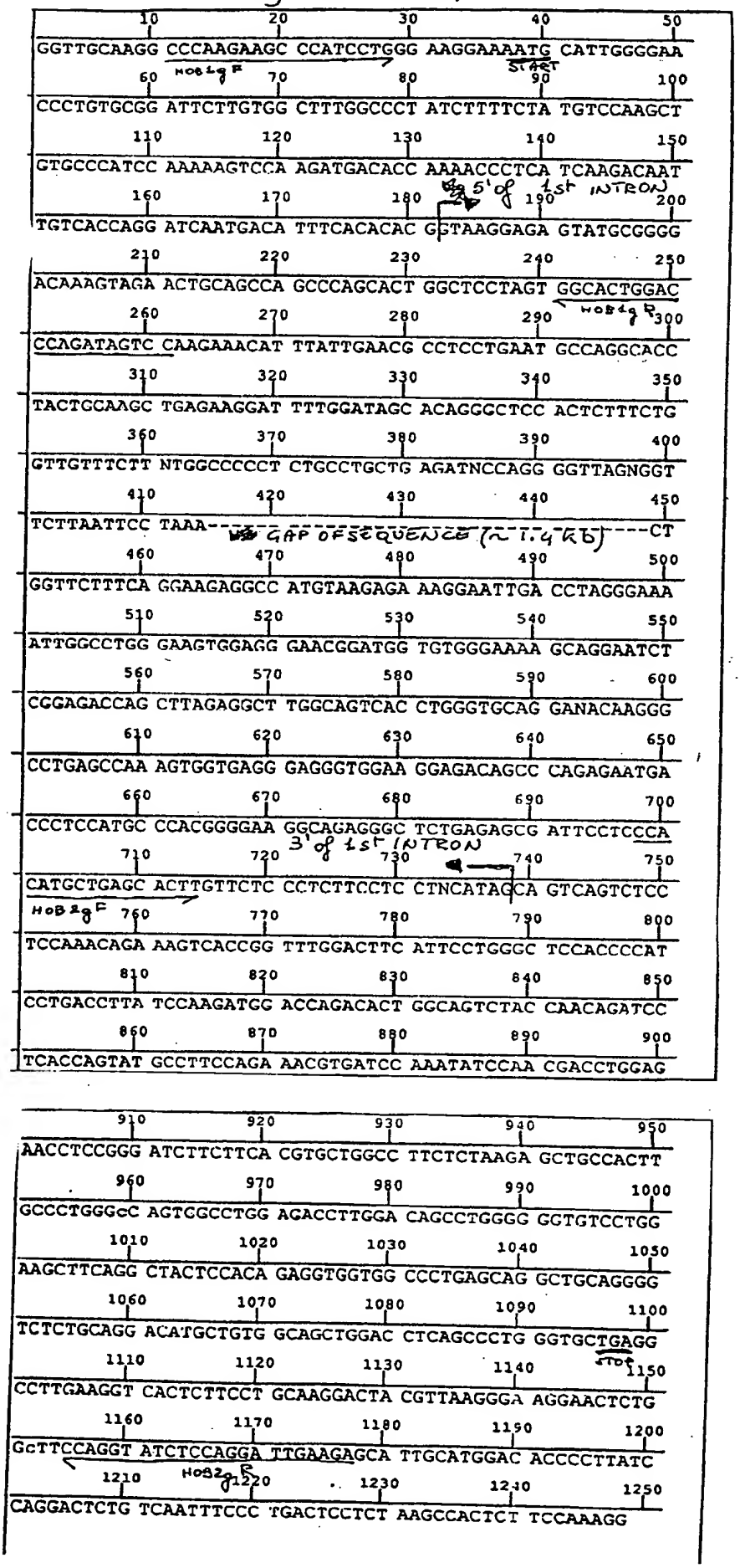

Triton X-100 – – – – +
Proteinase K – – + + +
Microsome – + – + +
preob
ob
31.0 kD
21.5 kD
14.3 kD

Figure 20A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HOB1gF
START
60 70 80 90 100
CCCTGTGCGG ATTCTTGTGG CTTTGGCCCT ATCTTTTCTA TGTCCAAGCT
110 120 130 140 150
GTGCCCATCC AAAAAGTCCA AGATGACACC AAAACCCTCA TCAAGACAAT
5' of 1st INTRON
160 170 180 190 200
TGTCACCAGG ATCAATGACA TTTCACACAC GGTAAGGAGA GTATGCGGGG
210 220 230 240 250
ACAAAGTAGA ACTGCAGCCA GCCCAGCACT GGCTCCTAGT GGCACTGGAC
HOB1gR
260 270 280 290 300
CCAGATAGTC CAAGAAACAT TTATTGAACG CCTCCTGAAT GCCAGGCACC
310 320 330 340 350
TACTGCAAGC TGAGAAGGAT TTTGGATAGC ACAGGCCTCC ACTCTTTCTG
360 370 380 390 400
GTTGTTTCTT NTGGCCCCCT CTGCCTGCTG AGATNCCAGG GGTTAGNGGT
410 420 430 440 450
TCTTAATTCC TAAA---GAP OF SEQUENCE (~1.4 KB)---CT
460 470 480 490 500
GGTTCTTTCA GGAAGAGGCC ATGTAAGAGA AAGGAATTGA CCTAGGGAAA
510 520 530 540 550
ATTGGCCTGG GAAGTGGAGG GAACGGATGG TGTGGGAAAA GCAGGAATCT
560 570 580 590 600
CGGAGACCAG CTTAGAGGCT TGGCAGTCAC CTGGGTGCAG GANACAAGGG
610 620 630 640 650
CCTGAGCCAA AGTGGTGAGG GAGGGTGGAA GGAGACAGCC CAGAGAATGA
660 670 680 690 700
CCCTCCATGC CCACGGGGAA GGCAGAGGGC TCTGAGAGCG ATTCCTCCCA
3' of 1st INTRON
710 720 730 740 750
CATGCTGAGC ACTTGTTCTC CCTCTTCCTC CTNCATAGCA GTCAGTCTCC
HOB1gF
760 770 780 790 800
TCCAAACAGA AAGTCACCGG TTTGGACTTC ATTCCTGGGC TCCACCCCAT
810 820 830 840 850
CCTGACCTTA TCCAAGATGG ACCAGACACT GGCAGTCTAC CAACAGATCC
860 870 880 890 900
TCACCAGTAT GCCTTCCAGA AACGTGATCC AAATATCCAA CGACCTGGAG
910 920 930 940 950
AACCTCCGGG ATCTTCTTCA CGTGCTGGCC TTCTCTAAGA GCTGCCACTT
960 970 980 990 1000
GCCCCTGGGCC AGTCGCCTGG AGACCTTGGA CAGCCTGGGG GGTGTCCTGG
1010 1020 1030 1040 1050
AAGCTTCAGG CTACTCCACA GAGGTGGTGG CCCTGAGCAG GCTGCAGGGG
1060 1070 1080 1090 1100
TCTCTGCAGG ACATGCTGTG GCAGCTGGAC CTCAGCCCTG GGTGCTGAGG
STOP
1110 1120 1130 1140 1150
CCTTGAAGGT CACTCTTCCT GCAAGGACTA CGTTAAGGGA AGGAACTCTG
1160 1170 1180 1190 1200
GCTTCCAGGT ATCTCCAGGA TTGAAGAGCA TTGCATGGAC ACCCCTTATC
HOB2gR
1210 1220 1230 1240 1250
CAGGACTCTG TCAATTTCCC TGACTCCTCT AAGCCACTCT TCCAAAGG

Figure 20B

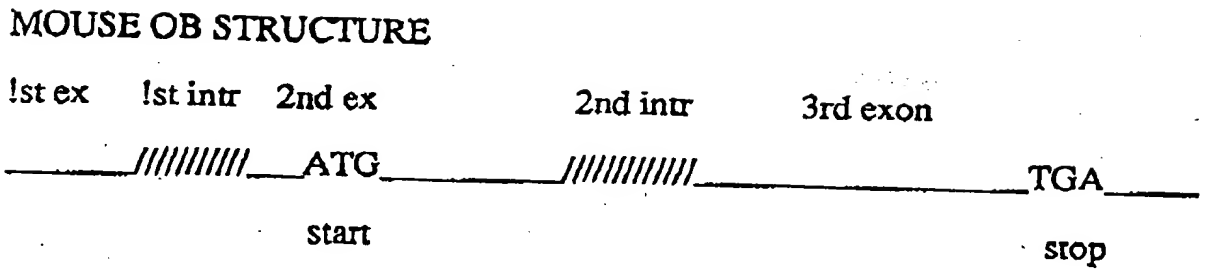

Figure 20c

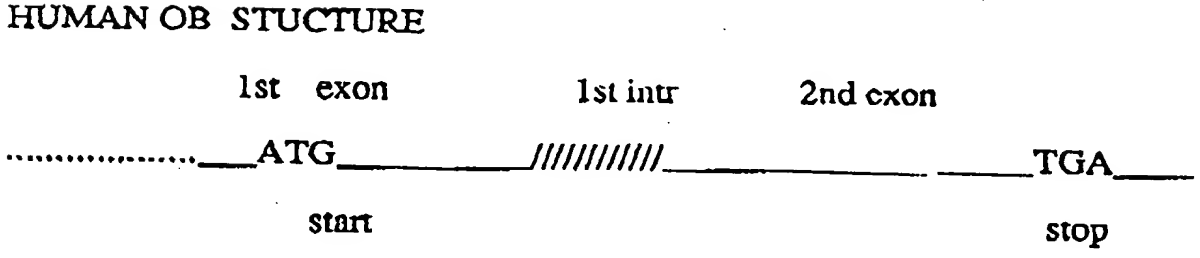

Figure 21A

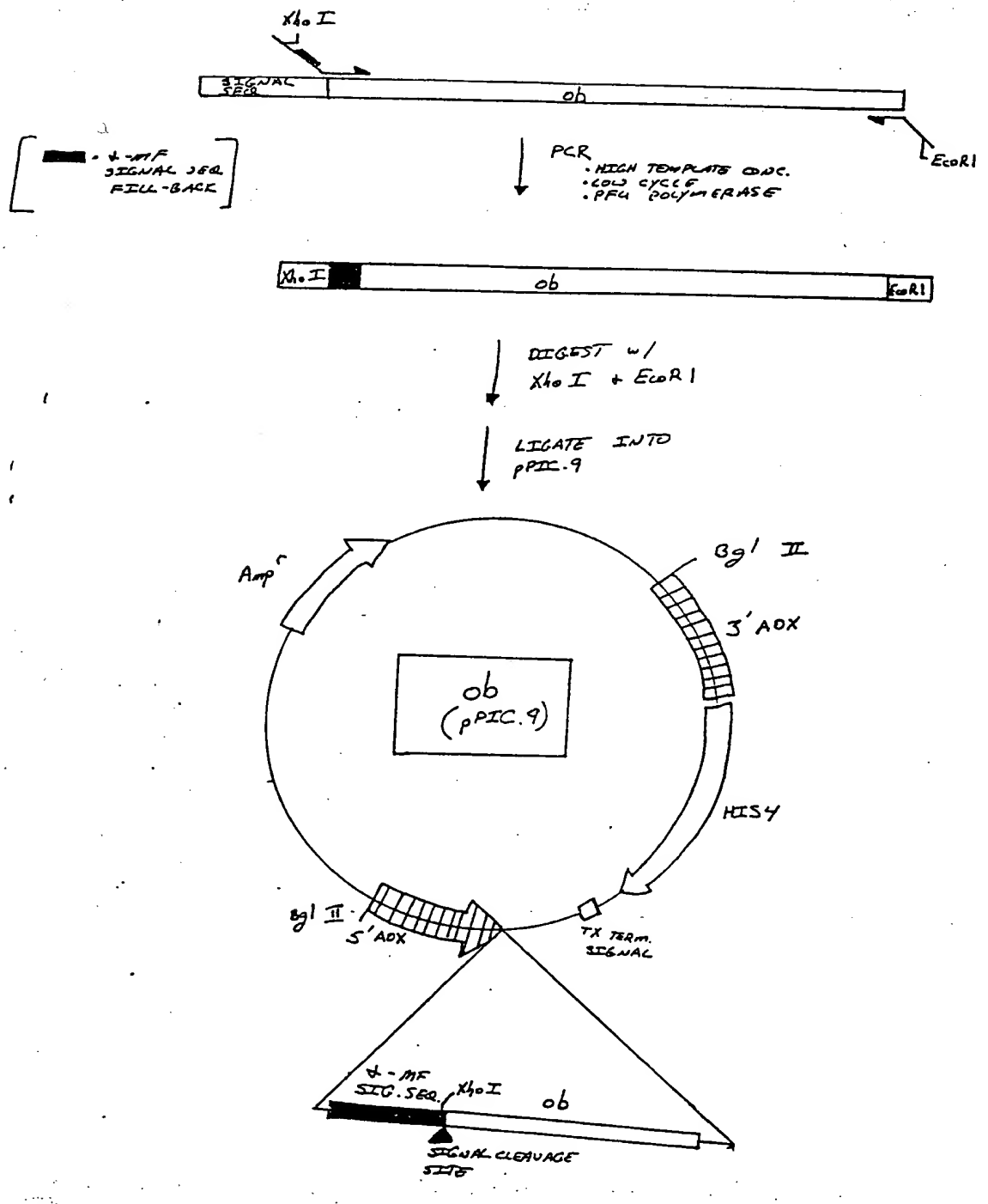
Xho I
SIGNAL SEQ
ob
EcoRI
α-MF SIGNAL SEQ. FILL-BACK
PCR
• HIGH TEMPLATE CONC.
• LOW CYCLE
• PFU POLYMERASE
Xho I
ob
EcoRI
DIGEST w/ Xho I + EcoR I
LIGATE INTO pPIC.9
Amp^r
Bgl II
3' AOX
ob (pPIC.9)
HIS4
Bgl II
5' AOX
TX TERM. SIGNAL
α-MF SIG. SEQ.
Xho I
ob
SIGNAL CLEAVAGE SITE

Figure 21 B

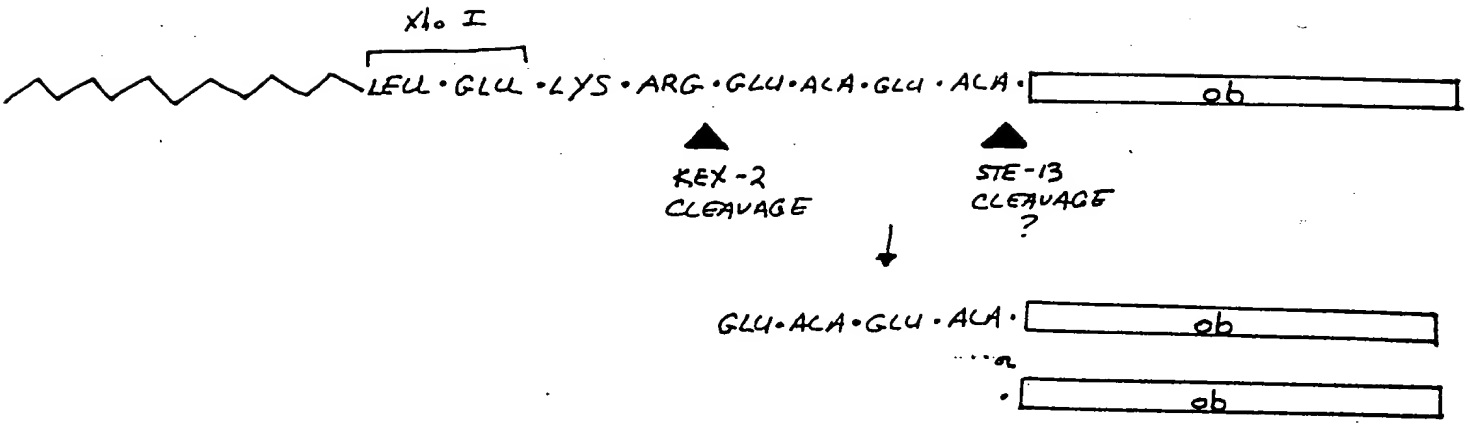

Figure 21 C

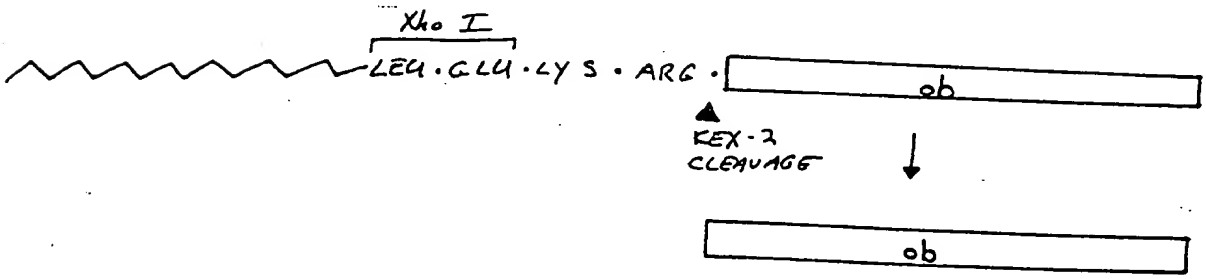

Figure 22A

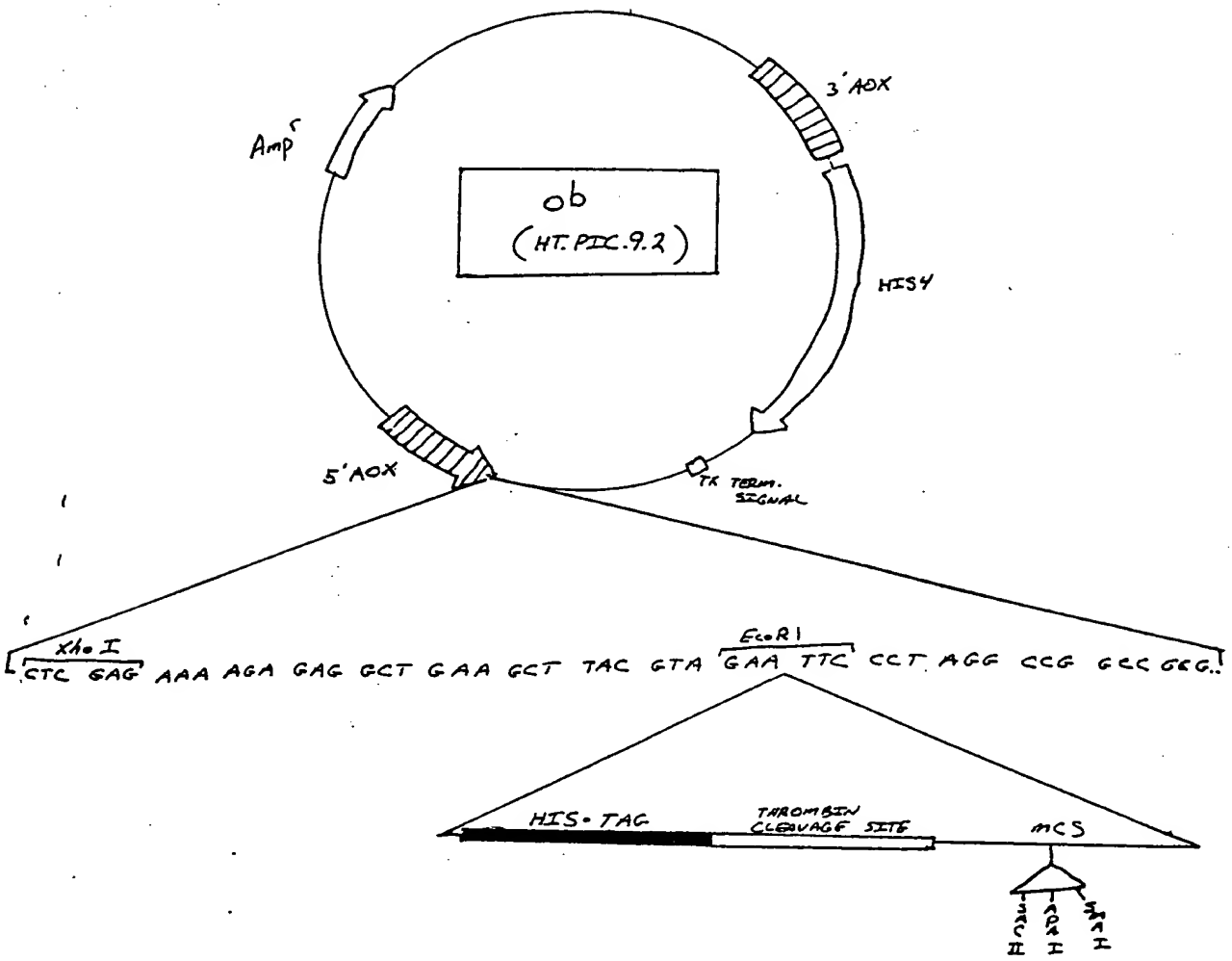
Amp^r
3'AOX
ob
(HT.PIC.9.2)
HIS4
5'AOX
TX TERM. SIGNAL
Xho I
CTC GAG AAA AGA GAG GCT GAA GCT TAC GTA GAA TTC CCT AGG CCG GCC GCG
EcoR I
HIS·TAG
THROMBIN CLEAVAGE SITE
MCS

Figure 22B

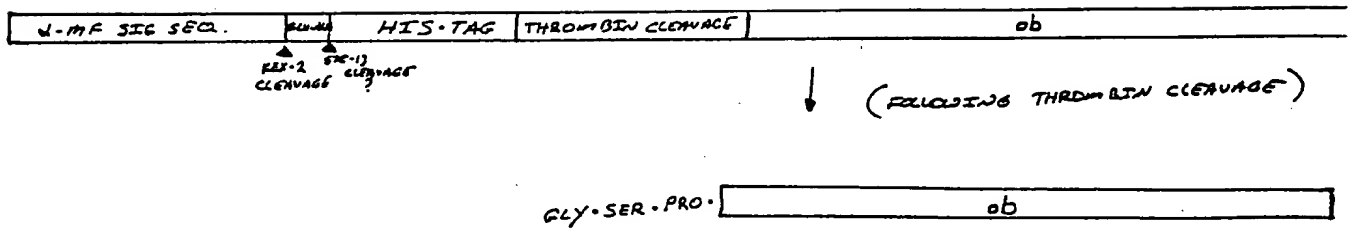
α-MF SIG SEQ.
HIS-TAG
THROMBIN CLEAVAGE
ob
KEX-2 CLEAVAGE
STE-13 CLEAVAGE
(FOLLOWING THROMBIN CLEAVAGE)
GLY-SER-PRO-
ob

Figure 23A.

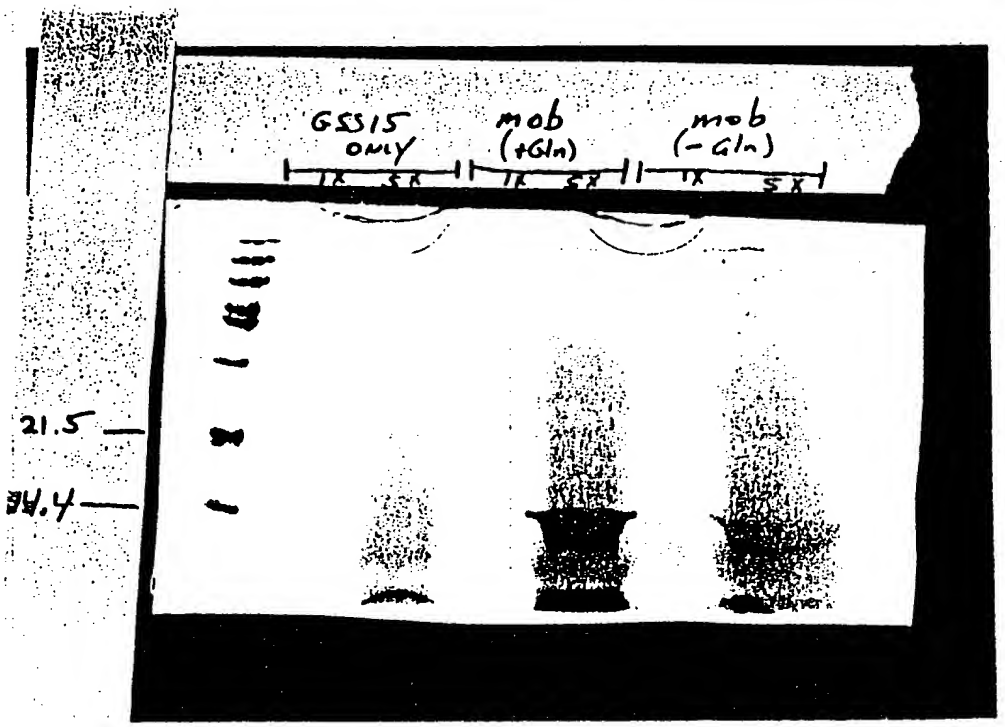

Figure 23B